THE EFFECT OF LIGHT REGIME ON THE PHOTOSYNTHETIC APPARATUS OF THE FRESHWATER RED ALGA *BATRACHOSPERMUM BORYANUM*

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ABSTRACT - Total irradiance appeared to control most pigment alterations in the freshwater red alga *Batrachospermum boryanum*. The only exception was a relative increase of phycoerythrin compared to phycocyanin in response to enhanced levels of green light under a natural tree canopy. However, this apparent complementary chromatic acclimatation was not confirmed by tests with color filters, either *in situ* or *in vitro*. Phycocyanin to phycoerythrin ratios tended to decrease as irradiance increased in laboratory studies. Phycobilisome size was greater in low light, field-acclimated plants. Average spacing between phycobilisomes was greater in high-light field populations. Photosynthesis in *B. boryanum* was negatively correlated to the phycocyanin to phycoerythrin ratio. Photosynthetic action spectra demonstrated a broad response to wavelength such that the alga was equally productive over most of the visible spectrum. This was also shown in the photosynthetic rates of plants grown under different wavelengths in the laboratory, where significant differences were not observed under either red, green, blue or neutral filters of equal light quantity.

RÉSUMÉ - L'irradiance globale a semblé contrôler la plupart des altérations pigmentaires de l'algue d'eau douce *Batrachospermum boryanum*. L'accroissement relatif de la phycoérythrine par rapport à la phycocyanine aux niveaux croissants de lumière sous une voûte d'arbres naturelle apparaît comme une exception. Cependant, des études avec les filtres colorés n'ont pas confirmé, ni *in situ* ni *in vitro*, ce qui semblait être une adaptation chromatique complémentaire. Dans les expériences de laboratoire la proportion de la phycoérythrine à la phycocyanine ainsi que les unités photosynthétiques avaient tendance à décroître au fur et à mesure que l'irradiance augmentait. Les plantes ayant poussé sous peu de lumière sur le terrain, possédaient une densité élevée de phycobilisomes. L'espacement moyen entre les phicobilisomes était plus grand parmi les populations existant dans des terrains à haute intensité lumineuse. Il y avait une corrélation négative entre la photosynthèse de *B. boryanum* et la proportion de la phycocyanine à la phycoérythrine. Le spectre d'action photosynthétique a démontré que l'algue était également productive dans la plupart du spectre visible. Ce phénomène se voyait aussi dans la vitesse de croissance des plantes cultivées en laboratoire sous des longueurs d'ondes différentes; des différences significatives n'étaient pas observées sous des filtres rouges, verts, bleus, ou neutres à flux lumineux identiques.

KEY WORDS : *Batrachospermum boryanum*, freshwater Rhodophyta, photoacclimation, phycobiliproteins.

INTRODUCTION

Studies on marine rhodophytes growing at various depths or using color mutants have indicated that light quantity alone affects pigments content (Dring, 1981; Ramus & van der Meer, 1983). However, in the freshwater environment, Thirb & Benson-Evans (1983) reported that alterations in pigment content and photosynthetic rates in *Lemanea sp.* occurred in response to changes in both light quantity and quality. It is clear that more taxa and additional environments need to be studied before it can be stated unequivocally that chromatic acclimatation does not occur in the Rhodophyta. This is particularly true since this phenomenon has been shown to take place in the Cyanophyta (e.g. MacColl & Guard-Friar, 1987).

Acclimation to total illumination in red algae typically involves increases in pigment content at lower irradiances. Little is known as to whether this involves changes in phycobilisome size or number (Gantt, 1990). Waaland *et al.* (1974) observed decreases in the number of phycobilisomes in *Griffithsia* at higher irradiance levels. Whether photoacclimation by change in phycobilisome number is universal in the Rhodophyta needs to be corroborated.

Batrachospermum boryanum Sirodot is a common inhabitant of temperate stream systems (Sheath & Burkholder, 1985) and, like many freshwater red algae, it is blue-green in color due to a relatively high level of phycocyanin (Honsell *et al.*, 1984). Rhodophyta growing in streams are frequently subjected to shading by over-hanging leaf canopy which results in significant seasonal variations in light quantity and quality. As the canopy becomes more dense, blue and red light decrease relative to other wavelengths (Federer & Tanner, 1966). This differs from the coastal marine environment which is usually enriched in green light and does not change appreciably over time (Ramus & van der Meer, 1983). Since most prior studies on chromatic acclimation have used marine red algae, the light conditions present in streams provide an excellent expansion of the photic conditions affecting pigment content and photosynthetic rates.

MATERIALS AND METHODS

Part I - Field studies 1987 and 1988

To establish different photoregimes in the field, a plexiglas chamber was designed after a model used by Triska *et al.* (1983): 1 m x 60 cm x 10 cm. It was divided equally into six, 10 cm wide chutes. The chutes allowed a uniform flow over the plants and manipulation of light quantity and quality.

The sites studied were situated in the Pawcatuck River drainage basin to ensure similar physico-chemical parameters other than light (Sheath &

Burkholder, 1985). Two plexiglas chambers were placed at each site. The open site was located on Chickasheen Brook in South Kingstown, R.I., U.S.A. (41°29'15"N, 71°32'45"W). The open site chambers were divided into five different sectors. One sector was left as an open control. The other four sectors had equal reduction of total irradiance to 15% of the open control: neutral density wire screen, red (peak 671 nm), green (peak 532 nm), or blue (peak 488 nm) acetate (Table II). The position of the various light sectors in each plexiglas chamber was determined by random selection.

Table I - Relative light energy at six different wavelengths over the course of the field studies. - Canopied Site. (Values are standardized to the means).

Wavelength (nm)	February	March	April	May	June
410 (violet)	0.95	1.07	1.05	1.02	1.00
488 (blue)	1.26	1.34	1.26	1.15	1.00
532 (green)	1.16	1.16	1.16	1.28	1.33
570 (yellow)	0.95	0.90	0.89	1.02	1.00
625 (orange)	0.95	0.81	0.89	0.89	1.00
671 (red)	0.74	0.72	0.74	0.64	0.67

Table II - Relative energy at six different wavelengths over the course of the field studies. - Filter tests at open site. (Values are standardized to the means).

Wavelength (nm)	Red	Green	blue	Neutral	Open Control
410 (violet)	0.00	0.00	0.29	0.97	1.01
488 (blue)	0.00	1.50	3.77	1.28	1.28
532 (green)	0.00	3.12	1.84	1.19	1.17
570 (yellow)	0.14	1.04	0.10	0.93	0.92
625 (orange)	2.73	0.00	0.00	0.88	0.89
671 (red)	3.14	0.35	0.00	0.75	0.73

The experiment began in early March and continued through the end of May 1987 and then was repeated from March to June 1988. This included the period of peak biomass for *Batrachospermum boryanum* (Sheath & Burkholder, 1985). Samples of *B. boryanum* were collected in triplicate at three-week intervals from all light sectors. A three-week period is more than adequate since light acclimation typically takes only several cell divisions (Levy & Gantt, 1988). The plants were promptly taken to the laboratory at each sampling for analysis.

Pigment analysis was performed according to the methods of Siegelman (pers. comm.). The samples were ground in liquid nitrogen, suspended in 0.055 M potassium phosphate buffer (KPi), 0.2 M NaCl (pH 6.8) and centrifuged at 10000 g for 5 min at 4°C. The raw phycobiliprotein extract (supernatant) was collected, the pellet was ground again and the procedure was repeated until no visible color remained in the supernatant. After extraction of phycobiliprotein pigments, the pellet was then resuspended in 90% acetone saturated with $MgCO_3$. Centrifugation was repeated and the chlorophyll *a* (chl *a*) extract (supernatant) was collected. The phycobiliprotein and chl *a* fractions were quantified using a spectrophotometer (Varian -

Model DMS-90). Equations and extinction coefficients were given by Siegelman & Kycia (1978).

Midday measurements of light quantity and quality were taken first through each of the various light screens at the open site on a sunny day and a cloudy day. A spectroradiometer (Biospherical - Model Mer-1010A) was used to measure light at six wavelengths: 410 nm (violet), 488 nm (blue), 532 nm (green), 570 nm (yellow), 635 nm (orange) and 671 nm (red). Light readings were then recorded at both the open and canopied streams at six different points at each site and three points along the length of each chamber (Tables I and II).

Measurements were taken near midday at the middle of each month on sunny days from September of 1987 through August of 1988. By combining meteorological data (% cloudiness and daylenght, National Weather Service - Warwick, R.I., U.S.A.) with actual light measurements at the stream sites, it was possible to obtain estimates of the total energy received by the plants in (mol m^{-2} d^{-1}) for both years of the field study (Table III and IV).

Table III - Total pigment content (mg g^{-1} fw), phycocyanin to phycoerythrin and phycobiliprotein to chlorophyll *a* ratios and light energy received by plants (mol m^{-2} d^{-1}) in 1987 and 1988 field studies.

Date	Energy	Total pigment	PC/PE	PBP/chl *a*
1987 - Canopied Site				
(3/25)	13.3	0.191	1.3	1.6
(4/16)	24.4	0.295	1.0	1.6
(5/4)	5.0	0.619	0.8	0.6
(5/27)	1.7	0.700	0.6	0.7
1988 - Canopied Site				
(3/30)	13.3	0.363	1.1	1.7
(4/18)	5.0	0.218	1.0	1.7
(5/18)	5.0	0.175	0.9	0.5
(6/1)	1.7	0.341	0.7	0.6
1987 - Open Site				
(3/25)	43.3	0.095	1.0	1.3
(4/16)	48.1	0.093	1.0	0.3
(5/4)	48.3	0.332	0.9	0.3
(5/27)	49.5	0.120	1.1	0.5
1988 - Open Site				
(3/30)	45.5	0.154	0.9	0.8
(4/18)	49.9	0.124	0.9	0.7
(5/18)	58.8	0.138	0.9	0.5
(6/1)	47.3	0.136	1.1	0.4

Table IV - Total pigment content (mg g^{-1} fw), phycocyanin to phycoerythrin and phycobiliprotein to chlorophyll *a* ratios and light energy received by plants (mol $m^{-2}d^{-1}$) in 1987 and 1988 field studies. (Filter tests at open site).

Date	Energy	Total pigment	PC/PE	PBP/chl *a*
1987 - Blue filter (Maximum transmittance - 488 nm)				
(3/25)	6.5	0.108	0.8	0.6
(4/16)	7.3	0.134	0.7	0.3
(5/4)	7.3	0.336	1.1	0.3
(5/27)	7.5	0.173	1.0	0.3
1988 - Blue Filter				
(3/30)	6.8	0.274	1.1	1.0
(4/18)	7.5	0.350	0.9	0.6
(5/18)	8.8	0.170	0.9	0.6
(6/1)	7.1	0.194	1.2	0.5
1987 - Red filter (Maximum transmittance - 671 nm)				
(3/25)	6.5	0.075	1.0	1.1
(4/16)	7.3	0.081	0.7	0.5
(5/4)	7.3	0.366	1.0	0.3
(5/27)	7.5	0.217	0.9	0.1
1988 - Red Filter				
(3/30)	6.8	0.290	1.1	1.7
(4/18)	7.5	0.251	0.7	0.6
(5/18)	8.8	0.175	1.0	0.5
(6/1)	7.1	0.217	1.3	0.6
1987 - Green Filter (Maximum transmittance - 532 nm)				
(3/25)	6.5	0.110	0.9	1.1
(4/16)	7.3	0.168	1.0	0.7
(5/4)	7.3	0.349	0.9	0.3
(5/27)	7.5	0.219	0.8	0.3
1988 - Green Filter				
(3/30)	6.8	0.162	0.9	1.5
(4/18)	7.5	0.171	0.8	0.7
(5/18)	8.8	0.150	1.0	0.5
(6/1)	7.1	0.184	1.1	0.3
1987 - Neutral Filter				
(3/25)	6.5	0.120	0.9	0.7
(4/16)	7.3	0.186	0.7	0.3
(5/4)	7.3	0.314	0.8	0.3
(5/27)	7.5	0.191	0.8	0.3
1988 - Neutral Filter				
(3/30)	6.8	0.316	0.9	0.9
(4/18)	7.5	0.238	0.9	0.3
(5/18)	8.8	0.224	0.6	0.3
(6/1)	7.1	0.220	1.0	0.3

Differences in means between populations were calculated based on the following: phycocyanin content (PC), phycoerythrin (PE), allophycocyanin (APC), chl *a*, PC/PE and total phycobiliproteins to chl *a* (PBP/chl *a*). A one-way analysis of variance (ANOVA) was performed using the Minitab computing system (Ryan *et al.*, 1976). Pearson-product moment correlations were calculated between total light energy received or the relative amount of green light and each of the various pigment parameters.

Part II - Lab Studies

A. Light saturation curves and photosynthetic action spectra for low and high light-acclimated populations of B. boryanum

Field samples of *B. boryanum* were collected from two different sites along the Chipuxet River. One of these was the canopied site used in the 1987 and 1988 field studies. At the time of collection, it had approximately 30% light transmitted at the streams surface relative to an open area immediately adjacent the stream (designated "low light"). The other site was located in South Kingstown, R.I. (41°28'45"N, 71°33'0"W) and had approximately 60% light transmitted at the stream surface ("high light").

All plants collected from the field were cleaned of epiphytes and debris and were transferred to Bold's Basic Medium (BBM). Photosynthetic rates were then obtained (mg O_2 min^{-1} g^{-1} fw) using an oxygen meter (Orbisphere - Model 2607). Oxygen readings were taken for 20 min light and 5 min darkness in order to obtain values for gross photosynthesis. Linearity of the readings was determined prior to the actual measurements. The light sources used were 122 cm, 40 Watt, wide spectrum fluorescent bulbs. Total irradiance was ajusted by applying layers of white mesh cloth to the outside of the glass vessels. The range of irradiances was 0 to 400 μmol m^{-2} s^{-1} and three replicate samples were used for both the high and low light-acclimated plants. Temperatures were maintained at 10°C.

Action spectra were run at a non-saturating irradiance of 20 μmol $m^{-2}s^{-1}$ based on methods of Dring (pers. comm.). Nineteen narrow band interference filters (Oriel) were used, ranging from 402 nm (violet) to 701 nm (red). The light source was a 24 V, 150 W Tungsten Halogen Lamp (Bell and Howell). The oxygen meter (Model 781-b-Strathkelvin) was linked to a microcomputer (BBC Master Series). A variable transformer (Zenith) was connected to the light source projector to ajust light to equivalent photon fluxes for each color filter used. The temperature was maintained at 10°C by using a water bath. Values for gross photosynthesis in replicates of four or five were obtained by using 15 min light periods, followed by 10 min of darkness for each of the nineteen wavelengths.

B. Pigment analysis and photosynthesis in B. boryanum acclimated to different light quantities and qualities

In the spring of 1989, samples of *B. boryanum* were collected from the canopied stream section of the Chipuxet River that was used for the field studies. The plants were transferred into 200 ml of BBM. Plants were acclimated for six weeks to seven different light regimes: 2.2, 4.8, 13.6 and 28.1

mol m^{-2} d^{-1} (25, 55, 155 and 320 μmol m^{-2} s^{-1}) white light and three different light qualities at 4.8 mol m^{-2} d^{-1} (55 μmol m^{-2} s^{-1}). The light quality regimes were established with colored cellophane as follows: red (peak 671 nm), green (532 nm) and blue (410-488 nm) (Table V). There were three replicate glass vessels for each light condition. Light measurements and pigment analyses were performed as outlined earlier after a six-week interval. Photosynthetic rates were obtained for all samples as outlined in Part II-A, in attempt to relate photosynthesis to pigmentation. Differences in pigment content and gross photosynthesis were tested among the conditions with ANOVA. Similarly, relative correlations were done as described earlier.

Table V - Relative light energy at six different wavelengths. (Lab. study). - (Values are standardized to the means).

Wavelength (nm)	Red	Green	blue	Neutral	Open Control
410 (violet)	0.00	0.54	2.48	0.85	0.85
488 (blue)	0.00	0.82	1.43	0.64	0.64
532 (green)	0.00	1.91	1.04	0.81	0.80
570 (yellow)	0.00	1.09	0.52	0.99	0.96
625 (orange)	1.80	0.82	0.26	1.69	1.73
671 (red)	4.20	0.82	0.26	1.02	1.04

Part III - Electron microscopy

Samples of *B. boryanum* were collected from the open and shaded stream segments that were used in Part II-A and fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), post fixed in 1% osmium tetroxide, dehydrated in a standard ethanol/propylene oxide series and embedded in Spurr's medium. The specimens were sectioned with a LKB-III ultramicrotome and stained with 5% uranyl acetate for 20 min and Reynolds lead citrate for 8 min. Fascicle cell chloroplasts were photographed with an electron microscope (JEOL - 12000 EX STEM) at 60,000 x magnification in replicates of ten. Measurements were made of phycobilisome diameter, spacing between adjacent phycobilisomes (taken from the center of each) and for spacing between thylakoid membranes. Averages and statistical differences were determined using ANOVA as stated previously.

RESULTS

Part I - Field studies 1987 and 1988

The open site had significantly higher irradiance values than the canopied site throughout the year (Fig. 1). All statistical differences in the field study were at the 95% confidence level unless otherwise noted. Variations between the sites were much greater when the leaf canopy was present from May to October. The maximum difference was approximately 1970 μmol m^{-2} s^{-1}. In contrast, the difference between the two sites in the month of February was only about 750 μmol m^{-1} s^{-1}. Peak irradiance occurred at the canopied site in April (ca. 800 μmol m^{-2} s^{-1}) just before the leaf canopy reappeared. The relatively small peaks seen at both sites in January (ca. 1850

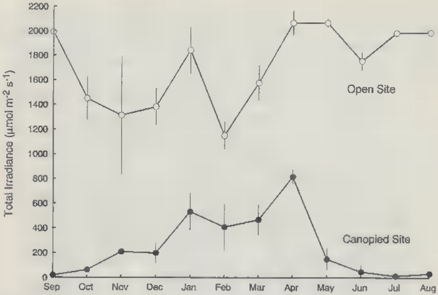

Fig. 1. - Total irradiance vs month at canopied and open sites (Error Bars = 1 standard deviation).

μmol m^{-2} s^{-1} at the open site and 530 μmol m^{-1} s^{-1} at the canopied site) were associated with increased light reflectance due to snow cover (Fig. 1).

The relative proportion of various light wavelengths did not change significantly through the year at the open site. However, at the canopied site there were significantly higher levels of green light and lower levels of red and blue light at the time of leaf canopy presence (Table I). In contrast, from February through April, the relative proportion of each wavelength approximated the values at the open site. For example, standardized values for green light rose from approximately 1.16 to 1.33 from April to June after remaining constant at 1.16 from February to April.

The total energy received by the plants (in mol m^{-2} d^{-1}) increased significantly over the course of the experimental period at the open site only in 1987 (Table III). In constrast, significant reductions were observed at the canopied site in late spring, when the leaves developed in both years. Energy estimates under the various light quality filters at the open site approximated values taken at the canopied site under developing leaves (April 16 to May 4, 1987) and (April 18 to May 18, 1988) (Tables III and IV).

Total pigment levels fluctuated throughout the experimental period in both the canopied and open site populations. These levels were significantly higher at the canopied site for most sampling times. Total pigment levels ranged from 0.075 to 0.700 mg/g fw for all samples (Tables III and IV). These relatively low values were due to the low dry wt./fresh ratio in *B. bor-*

yanum (c.f. 0.02) (Hambrook, pers. comm.). The PBP/chl *a* ratio was generally higher at the canopied site than at the open control, but it was significantly higher only on April 16 (1.6 to 0.3) and May 27 (0.7 to 0.5) in 1987 (Table III). This ratio decreased as total light increased in the open control samples for both years. In 1987 the mean ratios declined from 1.3 (March 25) to 0.5 (May 27). In 1988 the decline was from 0.8 (March 30) to 0.5 (June 1). Over the same period of time, total light at the open site increased from 43.3 to 49.9 mol m^{-2} d^{-1} in 1987 and from 45.5 to 47.3 in 1988. The negative correlation between the two variables was significant ($p < 0.01$) only in 1987. At the canopied site the PBP/chl *a* ratio decreased as total light declined, but the correlation was not significant in either year. In addition, the PBP/chl *a* ratio was negatively correlated to the relative amount of green light at this site but the correlation was significant only in 1988 (Tables I and III).

In 1987 total pigment content in the color filter tests peaked in all of these groups on May 4 and declined thereafter and the PBP/chl *a* ratios decreased over time (Table IV). In 1988 total pigment content peaked at different times in the various color tests. There was a general decline in the PBP/chl *a* ratio as in 1987. However, this ratio decreased significantly over time in most sample groups at each site for both years (Tables III and IV).

In both years of the field study the relative levels of PE and PC were either nearly equal or PC was slightly dominant at the beginning of the study (March 25 in 1987 and March 30 in 1988) (Table III). In the open control these pigment levels remained similar throughout the experimental period. In contrast, PE increased significantly compared to PC as the relative proportion of available green light increased under the tree canopy (Tables I and III). In 1987 the mean PC/PE ratio declined from 1.3 (March 25) to 0.6 (May 27). In 1988 the ratio declined from 1.1 (March 30) to 0.7 (June 1). Over the same period of time the relative amount of green light increased from 1.16 to 1.33 (Table I). There was a significant negative correlation between green light at the canopied site and the PC/PE ratio for both 1987 and 1988 ($p < 0.05$-0.01). No such correlation was observed at the open site. The correlation between total light energy (mol m^{-2} d^{-1}) and the PC/PE ratio at the canopied site was not significant in either year (Table III).

In the experimental light regimes at the open site, the relative levels of PE and PC fluctuated between PE and PC dominance (Table IV). APC consistently was significantly lower concentrations than the other pigments.

Part II - Lab Studies

A. Light saturation curves and photosynthetic action spectra for low and high light-acclimated populations of B. boryanum.

High light-acclimated plants had significantly greater gross photosynthetic rates than low light plants over the full range of irrandiances tested ($p < 0.05$). Maximum photosynthetic rates in the high and low light groups were 22.2 mg O_2 min^{-1} g^{-1} fw and 8.0 mg O_2 min^{-1} g^{-1} fw, respectively. Light saturation occurred at an irradiance of ca. 250 μmol $m^{-2}s^{-1}$ in both groups (data not shown).

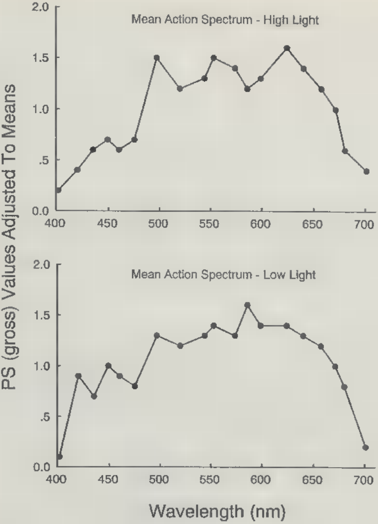

Fig. 2. - Mean photosynthetic action spectra for high and low light field-acclimated populations of *B. boryanum*.

The action spectra were not significantly different ($p < 0.05$) between high and low light-acclimated plants (Fig. 2). Maximum photosynthesis occurred over a broad range in the middle of the visible spectrum and significant differences between rates were seen only at each end of the spectrum. In the high light plants, the photosynthetic rates at wavelengths between 544 nm and 553 nm (green) and between 599 nm and 671 nm (orange-red), were significantly higher and those between 402 nm and 435 nm (violet-blue), 475 nm (blue) and 680 to 701 nm (red). In the low light plants, photosynthetic rates between 553 nm and 624 nm (green-orange) were significantly higher than those between 402 nm and 435 nm (violet-blue) and at 701 nm (red).

B. Pigment analysis and photosynthesis in B. boryanum acclimated to different light quantities and qualities

Pigment contents of *B. boryanum* plants acclimated to different light quantities and qualities in the laboratory were not significantly different ($p < 0.05$) in terms of PE, PC, APC or chl *a* under the different light regimes. Plants from the first (2.2 mol $m^{-2}d^{-1}$) (25 μmol $m^{-2}s^{-1}$). Neutral group has a significantly higher PC:PE ratio than those from the fourth (28.1 mol $m^{-2}d^{-1}$) (320 μmol $m^{-2}s^{-1}$). Neutral group (1.4 to 0.8) and there was a significant negative correlation between the PC:PE ratio and total energy received in the four different irradiance groups (table VI). Total energy received by the plants (mol $m^{-2}d^{-1}$) was in the range of values obtained for the canopied site *in situ* (Tables III and VI).

Table VI - Total pigment content (mg g^{-1} fw), phycocyanin to phycoerythrin and phycobiliprotein to chlorophyll *a* ratios and light energy received by plants (mol m^{-2} d^{-1}). (Lab. study - 6 week time interval).

Condition	Energy	Total Pigment	PC:PE	PBP:Chl *a*
Blue	4.8	0.169	1.3	0.9
Red	4.8	0.185	1.4	1.1
Green	4.8	0.123	1.5	1.9
Neutral (1)	2.2	0.181	1.4	0.9
Neutral (2)	4.8	0.221	1.3	0.9
Neutral (3)	13.6	0.109	1.0	1.4
Neutral (4)	28.1	0.084	0.8	0.5

Photosynthetic rates were greater at the higher acclimation irradiances (range 1.02-3.06 mg O_2 $min^{-1}g^{-1}$ fw and 10.0 to 60.7 mg O_2 min^{-1} mg^{-1} total pigments for Neutral (1) and Neutral (4), respectively). The rates did not vary significantly among plants acclimated to different light qualities at 4.8 mol $m^{-2}d^{-1}$ (55 μmol m^{-2} s^{-1}) but the following trends were observed: green light acclimated plants had higher photosynthetic rates that blue light plants (2.0 to 1.0 mg O_2 $min^{-1}g^{-1}$ fw and 16.2 to 8.9 mg O_2 min^{-1} mg total pigment). Likewise, green light acclimated plants had higher photosynthetic rates than red light plants whose rates were 1.5 mg O_2 $min^{-1}g^{-1}$ fw and 8.1 mg O_2 min^{-1} mg^{-1} total pigment. Photosynthesis was negatively correlated to the PC/PE ratio in all seven of the different light quantity and quality groups. The PC/PE ratio ranged from 0.83 to 1.46, while photosynthesis ranged from 1.0 to 3.1 mg O_2 $min^{-1}g^{-1}$ fw and 5.6 to 38.0 mg O_2 min^{-1} mg^{-1} total pigments.

Part III - Electron microscopy

In situ populations of *B. boryanum* at the canopied site had a significantly larger ($p < 0.05$) mean phycobilisome diameter (29 nm) than did those in the open site (26 nm). Average spacing between adjacent phycobilisomes was significantly greater in the open samples (83 nm) compared to the shaded ones (69 nm). Mean spacing between the thylakoid membranes was 84 nm for open plants and 62 nm for those at the canopied site. However, this difference was not significant.

DISCUSSION

Despite seasonal increases at the canopied site, the percentage of green light never approached that of the coastal marineenvironment (ca. 40%) (Dring, 1981). The proportion of green wavelengths under the tree canopy was also relatively low when compared to the artificial green regime at the open site. Nevertheless, the small increases in green light and decreases in red and blue light which occurred at the shaded site were in accord with findings of Federer & Tanner (1966) who observed energy maxima in the green and minima in the red under various types of plant canopies. There was essentially neutral filtering of branches at the shaded site in winter.

The negative correlation between the relative amount of green light and the PC/PE ratio observed at the canopied site appears to represent chromatic acclimation (Bogorad, 1975), a process that has not yet been conclusively shown to occur in the Rhodophyta (Gantt, 1990; Ramus, 1983). In *B. boryanum*, the specific phycobiliprotein pigment types present include R-PE, R-PC and allophycocyanin (Gantt, pers. comm.). Isolated R-PE has an absorbance at 532 nm that is more than two times greater than the absorbance of R-PC at the same green wavelength (MacColl & Guard-Friar, 1987). Therefore, an increase in PE relative to PC is ecologically advantageous under conditions where green light is enriched. However, much of the evidence from the field studies did not support chromatic acclimation in *B. boryanum* as follows: 1) similar changes among the artificial light quality tests at the open site; and 2) decline in the PBP/chl *a* ratio over time in most sample groups, regardless of light regime. It is possible that the phycobiliproteins were utilized as a nitrogen source for metabolism, particularly in the older populations (MacColl & Guard-Friar, 1987).

An apparent light quantity effect on pigmentation was the increase in the PBP/chl *a* ratio with decreasing light levels in the open control samples from 1987. Similar inceases have been documented in other red algae (Larkum & Barrett, 1983). Since phycobiliproteins are the primary light harvesting components for photosystem II in the Rhodophyta, an increase in the pigments at lower irradiance levels should theoretically increase light absorption (Gantt, 1990).

Photosynthesis versus irradiance curves for low and high light-acclimated field populations of *B. boryanum* indicated that in both light saturation was in the range of the 250 μmol m^{-2} s^{-1} irradiance value which Kremer (1983) determined for a European population of *Batrachospermum* sp. This relatively low light saturation value would favor growth under irradiances similar to those that were found at the shaded stream site in the winter and early spring (range ca. 200-530 μmol m^{-2}s^{-1}), the period of peak biomass for *B. boryanum*.

Photosynthetic action spectra most closely resembled those of marine rhodophytes that have relatively high levels of phycocyanin, such as *Porphyra umbilicalis* (Lüning & Dring, 1985), but the protosynthetic response to wavelength of *B. boryanum* was somewhat broader. Maximum photosynthesis occurred within the absorbance range of the phycobiliprotein pigments, although it was not possible to statistically distinguish individual peaks. The decline in photosynthesis in the red region of the spectrum was not as pronounced as that reported by Lüning & Dring (1985) for various marine rhodophytes. In some green and brown seaweeds with thick thalli, action spectra between 430 and 680 nm are almost flat (Lüning & Dring, 1985). It is possible that a similar "flattening" occurred in *B. boryanum*, but to a lesser extent. The broad photosynthetic response to wavelengths in *B. boryanum* indicated a high degree of flexibility in utilizing light of different qualities. This was consistent with the lack of significant differences in photosynthesis among the various light quality groups in the laboratory study, but contrasted with work done on *Porphyridium purpureum* by Gantt (1990). In that alga red light (> 660 nm) grown cells had a lower rate of oxygen evolution that those grown under neutral filtering of equal irradiance.

The greater number of phycobilisomes per unit area in low light acclimated field population of *B. boryanum* as compared to high plants is in agreement with the findings of Staehelin *et al.* (1978) and Waaland *et al.* (1974) for marine rhodophytes. The greater phycobilisome size in shaded populations of *B. boryanum* is not substantiated by previous electron microscopic examinations of Rhodophyta (MacColl & Guard-Friar, 1987). In the Cyanophyta evidence has been presented supporting light quality effects on phycobilisome size (Raps *et al.*, 1985; Siegelman & Kycia, 1982).

As in the field study, most of the evidence from the laboratory did not support light quality acclimation of the photosynthetic apparatus in *B. boryanum*. Unlike in the field study, PC/PE ratios changed in response to light quantity. The lower PC/PE ratios that were observed under the highest acclimation irradiance were contrary to the results of Waaland *et al.* (1974) who found increases in this ratio under high light conditions in the marine rhodophyte *Griffithsia pacifica*. The observation that the PBP/chl *a* ratio had decreased under higher irradiances was in accord with that study and with *in situ* populations at the open site in this study. In some marine cyanophytes, phycoerythrin has been preferentially utilized as a nitrogen source under low conditions (MacColl & Guard-Friar, 1987). This could account for the higher PC/PE ratio that was observed in *B. boryanum* under low light conditions in this study. Declines in PBP/chl *a* under high light may be due in part to photooxidation of the phycobiliprotein pigments (MacColl & Guard-Friar, 1987).

The finding that most pigment changes in *B. boryanum* were related to total irradiance is consistent with the current concepts of photoacclimation in the Rhodophyta (Gantt, 1990; MacColl & Guard-Friar, 1987). However, possible phycobiliprotein utilization for metabolism could influence pigment ratio changes under different light conditions. This phenomenon should be examined further in the freshwater environment.

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